

CLAIMS

What is claimed is:

1. A method for accumulating a translocated protein in a plant tissue comprising:

- 5 a) providing a plant having cells comprising a transgene comprising a protein translocation cassette encoding a protein having the general structure: SSP-TP-ER; wherein:
- 10 (i) SSP is a sporamin signal peptide;
- (ii) TP is a protein to be translocated; and
- (iii) ER is an endoplasmic reticulum retention peptide; and
- 15 b) growing the plant under conditions whereby the protein translocation cassette is expressed, and the translocated protein is accumulated in the plant tissues.

2. A method according to Claim 1 wherein the plant tissues are selected from the group consisting of leaves and seeds.

3. A method according to Claim 1 wherein the translocated protein is accumulated in the endoplasmic reticulum of the plant tissues.

20 4. A method according to Claim 1 wherein the translocated protein is accumulated in an amount greater than 1.2% of the total soluble protein.

5. A method according to Claim 1 wherein said translocated protein accumulates in an amount greater than 8.7% of the total soluble protein.

25 6. A method according to Claim 1 wherein the endoplasmic reticulum retention peptide has an amino acid sequence selected from the group consisting of SEQ ID NO:33 and SEQ ID NO:34.

7. A method for accumulating a translocated protein in a plant tissue comprising:

- 30 a) providing a plant having cells comprising a transgene comprising a protein translocation cassette encoding a protein having the general structure: SSP-TP; wherein:
- (i) SSP is a sporamin signal peptide; and
- (ii) TP is a protein to be translocated; and
- 35 b) growing the plant under conditions whereby the protein translocation cassette is expressed, and the translocated protein is accumulated in the plant tissues.

8. A method according to Claim 7 wherein the plant tissues are leaves.

9. A method according to Claim 7 wherein the translocated protein is accumulated in the apoplast of the plant tissues.

5 10. A method according to Claim 7 wherein the translocated protein is accumulated in an amount greater than 2.4% of the total soluble protein.

11. A method according to Claim 7 wherein the translocated protein is accumulated in an amount greater than 8.5% of the total soluble protein.

10 12. A method for accumulating a translocated protein in a plant tissue comprising:

a) providing a plant having cells comprising a transgene, the transgene comprising a protein translocation cassette encoding a protein having the general structure: SSP-SProP-TP; wherein;

15 (i) SSP is a sporamin signal peptide; and

(ii) TP is a protein to be translocated;

(iii) SProP is a sporamin pro-peptide; and

20 b) growing the plant under conditions whereby the protein translocation cassette is expressed, and the translocated protein is the plant tissues.

13. A method according to Claim 12 wherein the plant tissues are seeds.

14. A method according to Claim 12 wherein the translocated protein is accumulated in the vacuole of the plant tissues.

25 15. A method according to Claim 12 wherein the translocated protein is accumulated in an amount greater than 5.5% of the total soluble protein.

30 16. A method according to Claim 12 wherein the translocated protein is accumulated in an amount greater than 8.2% of the total soluble protein.

17. A method according to any one of claims 1, 7 or 12, wherein the protein translocation cassette further comprises:

a) suitable regulatory sequences selected from the group consisting of promoters, enhancers, terminators; and

35 b) optionally, a 6x histidine tag operably linked to C-terminus of the translocated protein.

18. A method according to claim 17, wherein the promoter is selected from the group consisting of:

- a) constitutive promoters;
- b) tissue-specific promoters;
- c) developmental stage-specific promoters;
- d) inducible promoters;
- 5 e) viral promoters;
- f) male germline promoters;
- g) female germline promoters;
- h) common germline promoters;
- i) chemically inducible promoters;
- 10 j) plant floral common germline promoters;
- k) plant vegetative shoot apical meristem promoters; and
- l) plant floral shoot apical meristem promoters.

19. A method according to Claim 18, wherein the constitutive promoter is selected from the group consisting of a CaMV 35S promoter, a nopaline synthase promoter, an octopine synthase promoter, a ribulose-1,5-bisphosphate carboxylase promoter, Adh1-based pEmu, Act1, SAM synthase promoter, a Ubi promoter and a chlorophyll a/b binding protein promoter.

20. A method according to Claim 18 wherein the tissue specific promoter is isolated from genes encoding the proteins selected from the group consisting of napin, cruciferin, beta-conglycinin, phaseolin, zein, oleosin, acyl carrier protein, stearyl-ACP desaturase, fatty acid desaturases, glycinin, Bce4, vicilin, and patatin.

21. A method according to any one of claims 1, 7 or 12, wherein the translocated protein is selected from the group consisting of:

- a) a transformation marker;
- b) a protein conferring a morphological trait;
- c) a non-enzyme protein;
- d) an enzyme; and
- 30 e) a silk-like protein.

22. A method according to Claim 21 wherein the silk-like protein is natural or recombinant.

23. A method according Claim 21 wherein the silk-like protein is derived from silks produced by *Bombyx mori* or *Nephila clavipes*.

24. A method according to claim 22 wherein the silk-like protein comprises the amino acid sequence as set forth in SEQ ID NO:7 and the amino acid sequence as set forth in SEQ ID NO:8.

25. A method according to Claim 22 wherein the silk-like protein comprises the amino acid sequences selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:5.

5 26. A method according to Claim 23 wherein the silk-like protein is DP-1B.

27. A method according to any one of claims 1, 7 or 12 wherein the plant is selected from the group consisting of food plants, non-food plants, arboreous plants, and aquatic plants.

10 28. A method according to claim 27, wherein the plant is selected from the group consisting of corn, wheat, barley, oats, sorghum, rice, rye, grasses, banana, soybean, rapeseed, sunflower, cotton, tobacco, alfalfa, *Arabidopsis*, sugar beet, sugar cane, canola, millet, beans, peas, flax, and forage grasses.

15 29. A translocation protein cassette encoding a protein having the general structure: SSP-TP-ER; wherein:

- (i) SSP is a sporamin signal peptide;
 - (ii) TP is a protein to be translocated; and
 - (iii) ER is an endoplasmic reticulum retention peptide selected from the group consisting of SEQ ID NO:33 and SEQ ID
- 20 NO:34.

30. A protein translocation cassette encoding a protein having the general structure: SSP-SProP-TP; wherein:

- (i) SSP is a sporamin signal peptide;
 - (ii) TP is a protein to be translocated; and
 - (iii) SProP is a sporamin pro-peptide.
- 25